not shown). Taken together these results suggest that p75 is the principle TNFR on T lymphocytes and is sufficient for TNF-mediated T-cell apoptosis.

Finally, we assessed the role of TNF and Fas-mediated apoptosis in the CD4 and CD8 T-cell subsets. We found that the *gld* mutation in FasL almost completely blocked TCR-induced apoptosis of sorted $CD4^+$ T cells but was incapable of preventing apoptosis of most $CD8^+$ T cells (Fig. 4*a*). By contrast, anti-TNF hardly protected $CD4^+$ T cells, but prevented TCR-induced death of most $CD8^+$ T cells (Fig. 4*b*). Similar results were obtained with *lpr* T cells (data not shown).

We have found that TNF mediates Fas-independent mature T-cell apoptosis and may account for peripheral deletion in *lpr* mice[10 15,24]. In contrast to mature T cells, blocking Fas and TNF had no effect on thymocyte death *in vitro* (data not shown). TNF caused death at later times than Fas and was transduced by p75. This suggests a physiological role for p75 which does not contain homology to the Fas 'death domain' and uses different signalling pathways from the p55 TNFR that mediates apoptosis of non-lymphoid cells[19 21,25,26]. We also found that Fas alone accounted for almost all $CD4^+$ T-cell death, whereas TNF caused most $CD8^+$ T-cell death. $CD8^+$ T cells may therefore use FasL primarily to kill target cells and may rely on the slower TNF pathway for autoregulatory apoptosis. Our findings may explain why Fas defects in mice and humans cause humorally mediated autoimmune disorders[27,28] and why the virally induced deletion of $CD8^+$ T cells occurs in *lpr* mice[24]. It will be important to determine how these two distinct molecular pathways of apoptosis mediate mature T-cell homeostasis in other autoimmune and infectious diseases. □

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1. Webb, S., Morris, C. & Sprent, J. *Cell* **63,** 1249–1256 (1990).
2. Rocha, B. & von Boehmer, H. *Science* **251,** 1225–1230 (1991).
3. Moskophidis, D., Lechner, F., Pircher, H. & Zinkernagel, R. M., *Nature* **362,** 758–782 (1993).
4. Lenardo, M. J. *Nature* **353,** 858–861 (1991).
5. Critchfield, J. M. *et al. Science* **263,** 1139–1143 (1994).
6. Alderson, M. R. *et al. J. exp. Med.* **181,** 71–77 (1995).
7. Dhein, J. *et al. Nature* **373,** 438–441 (1995).
8. Brunner, T. *et al. Nature* **373,** 441–444 (1995).
9. Ju, S. T. *et al. Nature* **373,** 444–448 (1995).
10. Russell, J. H., Rush, B., Weaver, C. & Wang, R. *Proc. natn. Acad. Sci. U.S.A.* **90,** 4409–4413 (1993).
11. Gillette-Ferguson, I. & Sidman, C. L. *Eur. J. Immun.* **24,** 1181–1185 (1994).
12. Lynch, D. H. *et al. Immunity* **1,** 365–371 (1994).
13. Nagata, S. *Semin. Immun.* **6,** 3–8 (1994).
14. Takahashi, T. *et al. Cell* **76,** 969–976 (1994).
15. Scott, D., Kisch, W. & Steinberg, A. *J. Immun.* **150** 664–672 (1993).
16. Jacob, C. O. & McDevitt, H. O. *Nature* **331,** 356–358 (1988).
17. Mariani, S. M., Matiba, B., Armandola, E. A. & Krammer, P. H. *Eur. J. Immun.* **24,** 3119–3123 (1995).
18. Smith, C. A., Farrah, T. & Goodwin, R. G. *Cell* **76,** 959–962 (1994).
19. Ramsdell, F. *et al. Int. Immun.* **6,** 1545–1553 (1994).
20. Smith, C. *et al. J. Immun.* **144,** 162–174 (1990).
21. Bigda, M. *et al. J. exp. Med.* **180,** 445–460 (1994).
22. Tartaglia, L., Ayres, T., Wong, G. & Goeddel, D. *Cell* **74,** 845–853 (1993).
23. Mansour, S. L., Thomas, K. R. & Capecchi, M. R. *Nature* **336,** 348–353 (1988).
24. Razvi, E., Jiang, Z., Woda, B. A. & Welsh, R. M. *Am. J. Path.* **147,** 79–91 (1995).
25. Clement, M.-V. & Stamenkovich, I. *J. exp. Med.* **180,** 557–567 (1994).
26. Schulze-Osthoff, X., Krammer, P. H. & Dröge, W. *EMBO J.* **13,** 4587–4596 (1994).
27. Theofilopoulos, A., Kofler, R., Singer, P. & Dixon, F. *Adv. Immun.* **46,** 61–109 (1989).
28. Fisher, G. *et al. Cell* **81,** 935–946 (1995).
29. Sheehan, K. C. F. *et al. J. exp. Med.* **181,** 607–617 (1995).

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Facilitation of *lin-12*-mediated signalling by *sel-12*, a *Caenorhabditis elegans S182* Alzheimer's disease gene

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THE *lin-12* and *glp-1* genes of *Caenorhabditis elegans* are members of the *lin-12/Notch* family of receptors for intercellular signals that specify cell fate[1,2]. By screening for suppressors of a *lin-12* gain-of-function mutation, we identified a new gene, *sel-12*, which appears to function in receiving cells to facilitate signalling mediated by *lin-12* and *glp-1*. The *sel-12* gene encodes a protein with multiple transmembrane domains, and is similar to S182, which has been implicated in early-onset familial Alzheimer's disease[3]. The high degree of sequence conservation suggests that the function of the SEL-12 and S182 proteins may also be conserved.

The *lin-12(d)* hypermorphic mutation *lin-12(n950)* causes a Multivulva phenotype characterized by the production of ectopic pseudovulvae[4,5]. We screened for non-Multivulva revertants after ethyl methanesulphonate mutagenesis[5] of *lin-12(n950)* hermaphrodites; two recessive suppressors, *ar131* and *ar133*, proved to be alleles of a new gene, *sel-12* (*sel* means suppressors and/or enhancer of *lin-12*). These *sel-12* alleles cause an incompletely penetrant, recessive egg-laying-defective (Egl) phenotype in a *lin-12*(+) background. Because *sel-12(ar131)* is viable, fertile and Egl *in trans* to a deficiency (data not shown), we also performed a screen for mutations that fail to complement the Egl defect of *sel-12(ar131)*. From a screen of 5,900 mutagenized haploid genomes we identified two additional *sel-12* alleles. One allele obtained in this screen, *sel-12(ar171)*, displays a completely penetrant Egl defect as a homozygote and *in trans* to a deficiency, suggesting that *sel-12(ar171)* strongly reduces *sel-12* function. This inference is supported by the molecular analysis described below, which indicated that the *ar171* lesion would result in a truncated protein product.

The Egl phenotype caused by *sel-12* mutations in a *lin-12*(+) background is reminiscent of the Egl phenotype caused by reducing *lin-12* activity (see Table 1 legend). However, a more general involvement of *sel-12* in *lin-12*- and *glp-1*-mediated cell-fate decisions becomes apparent when the phenotypes of *lin-12; sel-12* and *glp-1;sel-12* double mutants are analysed (Table 1). We examined the genetic interactions of *sel-12* with two *lin-12* hypomorphic mutations, with a *lin-12(d)* hypermorphic mutation, and with a *glp-1* hypomorphic mutation. In all cases we found that reducing *sel-12* activity reduces *lin-12* or *glp-1* activity. These genetic interactions are exemplified by the effects of *sel-12* on two *lin-12*-mediated decisions, the anchor cell/ventral uterine precursor cell (AC/VU) decision and vulval precursor cell (VPC) specification.

The AC/VU decision involves an interaction between two initially equivalent cells of the somatic gonad, Z1.ppp and Z4.aaa. In a given hermaphrodite, Z1.ppp and Z4.aaa interact so that one of these cells becomes the AC and the other a VU[7 9]. When *lin-12* activity is eliminated, both Z1.ppp and Z4.aaa become ACs (the '2 AC defect'), and when *lin-12* is activated, as in *lin-12(d)* mutants, both Z1.ppp and Z4.aaa become VUs

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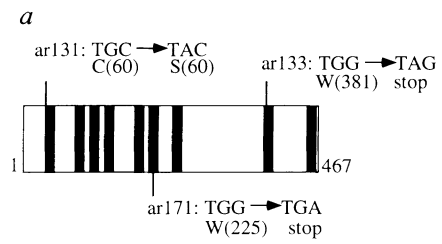

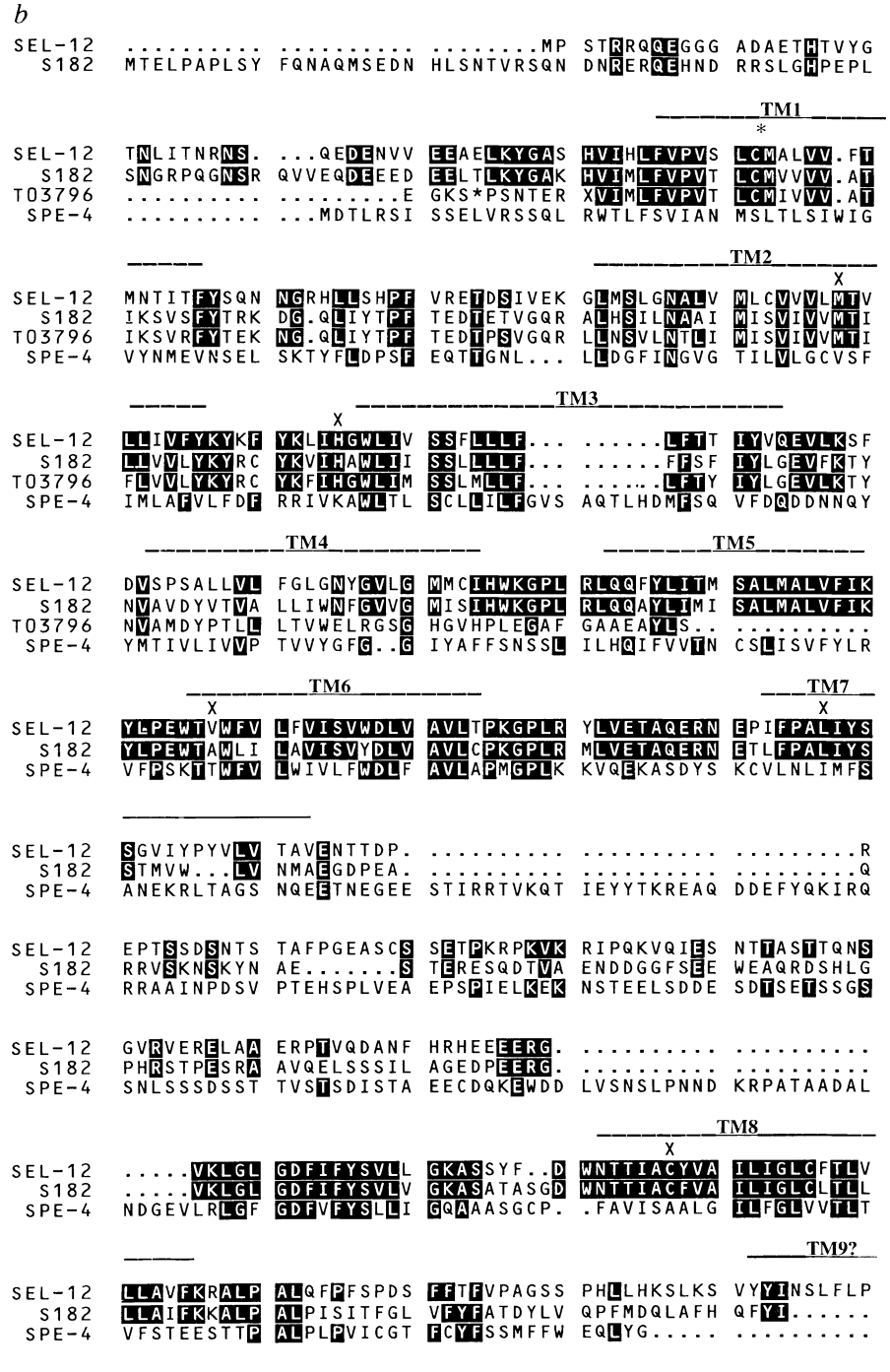

FIG. 1 *a*, Schematic representation of the SEL-12 protein and molecular lesions associated with three *sel-12* alleles. Filled rectangles indicate nine hydrophobic regions. Based on the Kyte–Doolittle algorithm, they are potential membrane-spanning domains. The fifth hydrophobic region contains only 18 amino acids and the sixth hydrophobic region contains a charged residue; however, these features are conserved in S182, so we infer that they are likely to be bona fide membrane-spanning domains. The ninth hydrophobic domain is not followed by a basic amino acid and is not conserved in S182 (although the carboxy terminus of S182 is relatively hydrophobic), so the inference that it is a membrane-spanning domain is more tentative. No potential signal sequence was identified. *b*, Predicted protein sequence of SEL-12 and its alignment with the predicted protein sequences of S182, T03796 and SPE-4. The PILEUP program of the GCG–Wisconsin package was used to create this alignment. Amino acids that are identical between SEL-12 and one or more of the other proteins are highlighted in black, and predicted transmembrane domains are overlined. The asterisk marks the conserved cysteine that is altered to a serine in *sel-12(ar131)*. S182 is the predicted protein of a gene associated with early-onset familial Alzheimer's disease[3], and the positions of the five mutations associated with the disease[3] are indicated (X). SEL-12 and S182 are 48% identical over a sequence of 460 amino acids. T03796 is a predicted protein from a partial cDNA isolated from an infant brain cDNA library[26]. This human cDNA clone was only partially sequenced on one strand[26]. SEL-12 and T03796 are 55% identical over a sequence of 104 amino acids. S182 and T03796 are highly similar, and it is unclear from the available sequence information if they correspond to the same gene. SPE-4 is the predicted protein of the *spe-4* gene of *C. elegans*, which is required for spermatogenesis[22]. SEL-12, S182 and T03796 appears to be much more closely related to each other than they are to SPE-4.

METHODS. We genetically mapped *sel-12* to the left of *unc-1 X:* from hermaphrodites of genotype *sel-12(ar131) dpy-3(e27)/unc-1(e538),* 1/36 Sel non-Dpy and 18/19 Dpy non-Sel recombinants segregated *unc-1*. To clone *sel-12*, we used the well-correlated genetic and physical maps in the *sel-12* region to identify cosmid clones that potentially carried the *sel-12* gene (ref. 27 and A. Coulson *et al.*, personal communication). We assayed pools and single cosmids for the ability to rescue the Egl defect of *sel-12(ar131)* hermaphrodites, using the plasmid pRF4 *(rol-6(su1006))* as a dominant cotransformation marker[28]. Ultimately, we found that pSpX4, containing a 3.5-kb *Spe*I/*Xho*I subclone of C08A12 (subcloned into KS Bluescript, Stratagene), completely rescued *sel-12(ar131)*. When this subclone was microinjected at a concentration of 10 μg ml^{-1} into *sel-12(ar131)* animals, all 6 lines demonstrated rescue of the Egl phenotype. When we attempted to obtain transgenic lines carrying pSpX4 in a *sel-12(+)* background using a concentration of 50 μg ml^{-1}, we obtained F_1 transformants but no stable lines, perhaps indicating some toxicity of this plasmid at higher concentrations. We used this genomic subclone to screen a cDNA library (kindly provided by Bob Barstead) and identified one class of clones of 1.5 kb in size. All subcloning, restriction digests, and library screening were done according to standard techniques. We sequenced both strands of the cDNA clone after generating systematic deletions using the Erase-a-base system (Promega). DNA sequencing was performed on double-stranded templates using Sequenase (US Biochemical). The cDNA contained both a poly(A) tail and a portion of the spliced leader sequence SL1 (ref. 29), suggesting it was a full-length clone. We confirmed the 5′ end of the cDNA by reverse transcription polymerase chain reaction (RT–PCR)[30]. The sequence of this full-length cDNA can be found through GenBank under accession number U35660. To identify the lesions associated with *sel-12* alleles we used PCR to amplify the *sel-12* genomic fragment from DNA isolated from the *sel-12* mutant strains using the primers DL103 (5′-TGTCTGAGTTACTAGTTTTCC-3′) and DLG3 (5′-GGAATCTGAAGCACCTGTAAGCAT-3′). A portion of this double-stranded amplification product was used as the template in a subsequent round of PCR using only the primer DL103, to generate a single-stranded template. Exon-specific primers were used to determine the entire coding sequence for all three alleles. For each allele, only one alteration in sequence was identified.

TABLE 1 *sel-12(ar171)* reduces *lin-12* and *glp-1* activity

(a) Enhancement of hypomorphic *lin-12* alleles by *sel-12(ar171)*

Genotype	% 2 ACs	% Ventral coelomocytes	Fertility	% L1 arrest*
Wild-type *C. elegans* var. Bristol strain N2	0	0	Yes	0
sel-12(ar171)unc-1(e538)	0 ($n=108$)	0 (0/17)	Yes	0 ($n=233$)
lin-12(n676n930); unc-1(e538)	30†	8 (1/12)	Yes	9 ($n=233$)
lin-12(n676n930); sel-12(ar171)unc-1(e538)	95 ($n=41$)	92 (12/13)	No	17 ($n=177$)
lin-12(ar170); unc-1(e538)	16 ($n=32$)	0 (0/32)	Yes	0 ($n=209$)‡
lin-12(ar170); sel-12(ar171)unc-1(e538)	98 ($n=47$)	0 (0/47)	Yes	0 ($n=111$)
lin-12(0)	100§	100§	No	10‖

(b) Suppression of a hypermorphic *lin-12* allele by *sel-12(ar171)*

Genotype	Number of VPCs adopting a vulval fate/hermaphrodite	% 0 AC
Wild-type *C. elegans* var. Bristol strain N2	3	0
lin-12(n950); unc-1(e538)	6 ($n=7$)	100
sel-12(ar171)unc-1(e538)	3 ($n=10$)	0 ($n=10$ 8)
lin-12(n950); sel-12(ar171)unc-1(e538)	2–4 ($n=8$)	89.5 ($n=5$ 7)

(c) Enhancement of *glp-1(e2141)* by *sel*-12(*ar*171)

Genotype	% Sterility in both gonad arms	% Sterility in one gonad arm
Wild-type *C. elegans* var. Bristol strain N2	0	0
glp-1(e2141); unc-1(e538)	8.5 ($n=259$)	4.0 ($n=259$)
sel-12(ar171)unc-1(e538)	0	0
glp-1(e2141); sel-12(ar170)unc-1(e538)	25 ($n=422$)	8.8 ($n=422$)

Most *lin-12*- and *glp-1*-mediated cell fate decisions appear normal in *sel-12(ar171)* mutants. However, the egg-laying defect of *sel-12(ar171)* hermaphrodites resembles the egg-laying defect of *lin-12* hypomorphic mutants[11]: *sel-12(ar131)* hermaphrodites leak occasional eggs and larvae, and like *lin-12* hypomorphic mutants, *sel-12* mutants have morphologically normal hermaphrodite-specific neuron (HSNs), sex muscles and VPC lineages. Egg laying is particularly sensitive to reduction in *lin-12* activity (ref. 11 and H. Wilkinson and I.G., unpublished observations). It is therefore possible that both *lin-12* and *sel-12* are required for an as yet unidentified cell fate decision(s) underlying the egg-laying defect. That *sel-12(ar171)* mutants do not display all of the defects associated with loss of *lin-12* function may indicate that *sel-12(ar171)* is not a null allele or *sel-12* function is partly redundant with the function of another gene. (*a*) Cell fate transformations were scored at 25 °C using criteria described in ref. 4 unless otherwise indicated. At 25 °C *lin-12(n676n930)* behaves like a hypomorph, whereas at 15 °C *lin-12(n676n930)* has mildly elevated *lin-12* activity[11]. Because *lin-12(n676n930); sel-12(ar171)* hermaphrodites are sterile at 25 °C, we shifted fertile *lin-12(n676n930); sel-12(ar171)* hermaphrodites from 15 to 25 °C so that their progeny could be scored for cell fate transformations and other defects. *lin-12(ar170)* behaves like a hypomorph for the AC/VU decision (J. Hubbard and I.G., unpublished observations). In strains containing *lin-12(ar170)*, cell fate transformations were scored in hermaphrodites raised at 20 °C, other defects were scored in the progeny of hermaphrodites grown at 20 °C and shifted to 25 °C. 2 ACs (%): in *lin12(0)* mutants, both Z1.ppp and Z4.aaa become ACs, so *lin-12(0)* hermaphrodites have two ACs; in *lin-12(d)* mutants such as *lin-12(n950)*, both Z1.ppp and Z4.aaa become VUs, so *lin-12(d)* hermaphrodites have 0 ACs. The number of anchor cells was scored in the L3 stage using Nomarski microscopy. For all genotypes, hermaphrodites either had one or two ACs. Ventral coelomocytes: the fates of two pairs of cells, M.d(l/r)pa and M.v(l/r)pa are affected by mutations in *lin-12*. In wild type, the ventral pair of cells gives rise to one sex myoblast and one body muscle; the dorsal pair gives rise to coelomocytes. In *lin-12(0)* animals, the ventral pair as well as the dorsal pair gives rise to coelomocytes, so that *lin-12(0)* hermaphrodites have extra ventral coelomocytes; in *lin-12(d)* animals, both pairs of cells give rise to sex myoblasts/body muscles. The presence of ventral coelomocytes was scored in the L3 stage. For all genotypes, the absence of ventral coelomocytes suggests that the sex myoblast was specified normally (see ref. 4). Fertility: fertility was scored by the appearance of eggs either on the plate or inside the hermaphrodite and the ability to propagate the strain. L1 arrest: full viability requires activity of *lin-12* or a related gene, *glp-1*. *lin-12(0) glp-1(0)* double mutants display a fully penetrant L1 arrest phenotype and a Lag phenotype characterized by specific cell fate transformations[23]. *lin-12(0)* single mutants display a low penetrance L1 arrest phenotype and a somewhat lower penetrance Lag phenotype[23]. Single gravid hermaphrodites were placed on a plate at 25 °C. Most of the hermaphrodites were completely egg-laying defective and laid no eggs; some *lin-12(n676n930)* animals released a few eggs or larvae before turning into 'bags of worms', in which case the hermaphrodite was transferred after a day. Because *lin-12(n676n930)* animals can grow slowly at 25 °C, L1 arrested animals were scored for 3 days after all the eggs had hatched. Arrested L1 animals were spotchecked for the presence of Lag phenotypes using Nomarski microscopy. Some arrested L1 animals of each genotype displayed Lag phenotypes (data not shown). (*b*) Animals were grown at 20 °C. VPC fates were scored by determining the cell lineages of P3.p–P8.p in each animal (Table 2 and data not shown). The number of ACs were scored as described above. For all genotypes, hermaphrodites had either 0 or 1 AC. (*c*) *glp-1(e2141ts)* is weakly hypomorphic at 20 °C and essentially wild type at 15 °C (ref. 24). Strains containing *glp-1(e2141)* were maintained at 15 °C; fertile adults grown at 15 °C were placed at 20 °C, and their progeny grown at 20 °C were scored for sterility. Other strains were maintained continuously at 20 °C. *glp-1* activity controls the decision of germline nuclei between mitosis and meiosis (refs 24, 25 and L. W. Berry and T. Schedl, personal communication). GLP-1 is thought to be the receptor for the inductive signal from the distal tip cells of the somatic gonad that promotes germline mitosis (and/or inhibits meiosis)[7]. When *glp-1* activity is eliminated, germline nuclei enter meiosis.[25]. Hermaphrodites of each genotype were scored for sterility in one or both gonad arms in the dissecting microscope. Several sterile or half-sterile individuals were examined by Nomarski microscopy, and sterile gonad arms were found to have the characteristic Glp phenotype (data not shown).

* Some L1-arrested animals were examined for Lag phenotypes: lack of an anus and rectum, lack of an excretory cell, and a twisted nose. Those phenotypes were observed for all genotypes where L1-arrested animals were identified.

† See ref. 11.

‡ *lin-12(ar170)* (not *unc-1*).

§ *lin-12(n137n720);* see ref. 4.

‖ *lin-12(n941);* see ref. 23.

(the '0 AC defect')[4,10]. Two observations indicate that *sel-12* reduces *lin-12* activity in Z1.ppp and Z4.aaa. First, *sel-12* dramatically enhances the penetrance of the 2 AC defect of *lin-12* hypomorphs (Table 1*a*). For example, 30% of *lin-12(n676n930)* hermaphrodites have 2 AC[11], whereas essentially all *lin-12(n676n930); sel-12(ar171)* have 2 ACs. Second, *sel-12* partly suppresses the 0 AC defect caused by LIN-12 activation (Table 1*b*). For example, all *lin-12(n950)* hermaphrodites lack an AC, whereas 10% of *lin-12(n950); sel-12(ar171)* hermaphrodites have an AC.

Each of the six VPCs, P3.p–P8.p, has the potential to adopt one of two vulval fates, termed primary (1°) and secondary (2°) or a non-vulval fate, termed tertiary (3°) (refs 12, 13). Normally, P5.p, P6.p and P7.p adopt vulval fates, in a 2°–1°–2° pattern[14]. This pattern is the outcome of the integration of two signalling inputs: a *let-60* Ras-mediated inductive signal from the AC induces vulval fates, and a *lin-12*-mediated lateral signal between VPCs prevents adjacent VPCs from adopting the 1° fate (reviewed in ref. 15). The *let-60* Ras-mediated inductive signal may cause expression or activation of the lateral signal[16,17], which activates LIN-12 to cause a VPC to adopt the 2° fate[3,18,19].

Reducing *sel-12* activity reduces *lin-12* activity in lateral signalling that specifies the 2° fate of VPCs. First, *sel-12* reduces the effect of activated LIN-12 in the VPCs: all VPCs adopt the

TABLE 2 *sel-12(ar171)* plays a role in the receiving cells

Genotype	Expression of 2° fate/total						VPCs adopting a 2° fate/hermaphrodite (%)
	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	
lin-12(n950)	7/7	7/7	7/7	7/7	7/7	7/7	100
lin-12(n950); sel-12(ar171)	0/8	1/8	4/8*	8/8	6/8	2/8†	52
lin-12(n950)	X	11/11	X	X	X	X	100
lin-12(n950); sel-12(ar171)	X	3/10	X	X	X	X	30

Animals were maintained at 20 °C. Early L2 hermaphrodites (as judged by the size of the gonad) were chosen for laser ablation studies. The fates of the VPCs have not been determined at this time; the VPCs become determined many hours later, in the L3 stage[13]. P3.p and P5.p–P8.p were killed with a laser microbeam; the success of this operation was verified 2–3 h later. The following day, the operated animals were mounted for Nomarski microscopy so that the cell lineage of P4.p could be observed directly. In both operated and unoperated animals, vulval fates were scored by directly observing the cell lineage of each VPC. The operated animals were observed until the early L4 stage, to ensure that no divisions were missed.

X indicates cell killed by a laser microbeam. Numbers in each column correspond to the proportion of times a given VPC was observed to adopt the 2° fate (criteria as in ref. 19). All VPCs that did not undergo 2° fates underwent 3° or non-vulval fates, with three exceptions: *, in 1/8 animals examined, P5.p underwent a hybrid (2°/3°) lineage; †, in 2/8 animals examined, P8.p underwent a hybrid (2°/3°) lineage.

2° fate in *lin-12(n950)* hermaphrodites, but only half of the VPCs adopt the 2° fate in *lin-12(n950); sel-12(ar171)* hermaphrodites (Tables 1*b* and 2). Second, *sel-12* reduces lateral signalling that occurs upon activation of *let-60* Ras. We analysed VPC lineages (data not shown) in *let-60(n1046)* hermaphrodites, in which Ras has been activated by a codon 13 mutation[20,21], and in *let-60(n1046); sel-12(ar171)* hermaphrodites. Lateral signalling appears to occur normally in *let-60(n1046)* hermaphrodites, as adjacent VPCs do not adopt the 1° fate (0 of 20 pairs of induced VPCs). In contrast, adjacent VPCs sometimes adopt the 1° fate in *let-60(n1046); sel-12(ar171)* hermaphrodites (4 of 18 pairs), implying that reducing the activity of *sel-12* reduces lateral signalling. Finally, some VPCs adopt the 2° fate in *lin-12(n676n930)* hermaphrodites[11]. In contrast, VPCs do not adopt the 2° fate in *lin-12(n676n930); sel-12(ar171)* double mutants (data not shown), although we have not tested whether this effect is due to the presence of a second AC.

The genetic interactions of *sel-12* with *lin-12* imply a function for *sel-12* in signalling and/or receiving cells during lateral specification. We have tested whether *sel-12* functions in the receiving end of *lin-12*-mediated cell–cell interactions by performing cell ablation experiments (Table 2). We reasoned that, if all VPCs but one were ablated with a laser microbeam, the fate of the isolated VPC would reflect its intrinsic level of *lin-12* activity in the absence of lateral signal. Thus, in *lin-12(n950)* hermaphrodites, an isolated VPC adopts the 2° fate (Table 2), suggesting that it has a high level of ligand-independent activation of LIN-12 in the VPCs[10]. If *sel-12* were to function in one VPC to lower *lin-12* activity in another, then in *lin-12(n950);sel-12(ar171)* hermaphrodites an isolated VPC should also adopt the 2° fate. However, if *sel-12* were to function within a VPC to lower its *lin-12* activity, then in *lin-12(n950);sel-12(ar171)* hermaphrodites an isolated VPC should instead adopt the 3° fate. We observed that in *lin-12(n950); sel-12(ar171)* hermaphrodites, an isolated P4.p often adopts the 3° fate (Table 2), implying that *sel-12* functions within a VPC to lower *lin-12* activity.

We cloned *sel-12* by transformation rescue (Fig. 1 legend), and determined the nucleotide sequence of a full-length cDNA (Genbank accession number U35660). The predicted SEL-12 protein contains multiple potential transmembrane domains (Fig. 1), consistent with its SEL-12 function as a receptor, ligand, channel or membrane structural protein. The SEL-12 protein is evolutionarily conserved. Database searches revealed a high degree of similarity to a sequence of a partial complementary DNA from human brain present on clone T03796, and a low degree of similarity to SPE-4, a protein required for *C. elegans* spermatogenesis[22]. In addition, SEL-12 is highly similar to S182, which, when mutant, has been implicated in familial early-onset Alzheimer's disease[3]. The predicted protein sequences of SEL-12, T03796, SPE-4 and S182 are aligned in Fig. 1.

Many different cell fate decisions are specified by *lin-12/Notch* genes in *C. elegans* and *Drosophila*, and in both organisms some of these decisions are critical for neurogenesis. The genetic analysis described here indicates that *sel-12* facilitates *lin-12*-mediated reception of intercellular signals. SEL-12 might be directly involved in *lin-12*-mediated reception, functioning for example as a co-receptor or as a downstream effector that is activated upon LIN-12 activation. Alternatively, *sel-12* may be involved in a more general cellular process such as receptor localization or recycling and hence influence *lin-12* activity indirectly. Although the remarkable conservation of SEL-12 and S182 does not provide any immediate indication of the function of S182 in the Alzheimer's disease process, it is striking that 4 of the 5 mutations found in affected individuals alter amino acids that are identical in SEL-12 and S182 (see Fig. 1). The powerful tools of classical and molecular genetic studies in *C. elegans*, including the ability to identify extragenic suppressors and to generate transgenic lines containing engineered genes, can now be brought to bear on fundamental issues of SEL-12/S182 structure and function. □

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1. Greenwald, I. *Curr. Opin. Genet. Dev.* **4,** 556–562 (1994).
2. Artavanis-Tsakonas, S., Matsuno, K. & Fortini, M. *Science* **268,** 225–268 (1995).
3. Sherrington, R. *et al. Nature* **375,** 754–760 (1995).
4. Greenwald, I., Sternberg, P. & Horvitz, H. R. *Cell* **34,** 435–444 (1983).
5. Ferguson, E. L. & Horvitz, H. R. *Nature* **110,** 259–267 (1985).
6. Brenner, S. *Genetics* **77,** 71–94 (1974).
7. Kimble, J. & Hirsh, D. *Devl Biol.* **81,** 208–221 (1979).
8. Kimble, J. *Devl Biol.* **87,** 286–300 (1981).
9. Seydoux, G. & Greenwald, I. *Cell.* **57,** 1237–1245 (1989).
10. Greenwald, I. & Seydoux, G. *Nature* **346,** 197–199 (1990).
11. Sundaram, M. & Greenwald, I. *Genetics* **135,** 755–763 (1993).
12. Sulston, J. & White, J. *Devl Biol.* **78,** 577–597 (1980).
13. Sternberg, P. & Horvitz, H. R. *Cell* **44,** 761–772 (1986).
14. Sulston, J. & Horvitz, H. R. *Devl Biol.* **56,** 110–156 (1977).
15. Horvitz, H. R. & Sternberg, P. W. *Nature* **351,** 535–541 (1991).
16. Simske, J. S. & Kim, S. K. *Nature* **375,** 142–146 (1995).
17. Tuck, S. & Greenwald, I. *Genes Dev.* **9,** 341–357 (1995).
18. Sternberg, P. W. *Nature* **335,** 551–554 (1988).
19. Sternberg, P. W. & Horvitz, H. R. *Cell* **58,** 679–693 (1989).
20. Beitel, G. J., Clark, S. G. & Horvitz, H. R. *Nature* **348,** 503–509 (1990).
21. Han, M. & Sternberg, P. W. *Cell* **63,** 921–931 (1990).
22. L'Hernault, S. W. & Arduengo, P. M. *J. Cell Biol.* **119,** 55–68 (1992).
23. Lambie, E. & Kimble, J. *Development* **112,** 231–240 (1991).
24. Priess, J. R., Schnabel, H. & Schnabel, R. *Cell* **51,** 601–611 (1987).
25. Austin, J. & Kimble, J. *Cell* **51,** 589–599 (1987).
26. Khan, A. S. *et al. Nature Genet.* **2,** 180–185 (1992).
27. Coulson, A., Waterston, J., Kiff, J., Sulston, J. & Kohara, Y. *Nature* **235,** 184–186 (1988).
28. Mello, C. C., Kramer, J. M., Stinchcomb, D. T. & Ambros, V. A. *EMBO J.* **10,** 3959–3970 (1991).
29. Krause, M. & Hirsh, D. *Cell* **49,** 753–761 (1987).
30. Ohara, O., Dorit, R. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **86,** 5673–5677 (1989).

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